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ORIGINAL ARTICLE

Novel usage of intraocular pressure-lowering drugs as wound-healing inhibitors after trabeculectomy with cell culture and animal models

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Abstract Inhibiting the healing of wounds to ensure that the aqueous humor can drain into the scleral space unimpeded and form a filtering bleb plays a crucial role in determining the success rate of glaucoma surgery. The aim of this study was to investigate the novel use, with cell culture and animal models, of some commercial intraocular pressure (IOP)-lowering drugs in inhibiting the healing of fibroblast wounds. The Tenon's fibroblasts of rabbits were cultured to evaluate 13 IOP-lowering drugs for cellular proliferation, collagen formation, and migration. These were measured using [^{3}H]thymidine and [^{3}H]proline uptake, and Transwell chambers. A preservative of benzalkonium chloride (BAK) was initially used, with 0.02% as a maximal original concentration. All of the drugs and the BAK were diluted from original commercial concentrations to 1/10, 1/100, and 1/1000. The more inhibitive drugs screened from the cell cultures were then selected for further short-term application during and after trabeculectomy surgeries had been performed on the rabbits. Expression of the proliferative cell nuclear antigen was immunohistochemically examined 3 and 7 days after surgery. The results revealed that the inhibitive effects of BAK in cellular [^{3}H]thymidine and [^{3}H]proline uptake, and cellular migration were only evident at 0.002% concentrations. Based on the results of the cell cultures, timolol, latanoprost, and unoprostone exhibited a greater inhibitory effect than

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the other drugs. Moreover, the animal studies showed that latanoprost and unoprostone significantly suppressed the positive expression of proliferative cell nuclear antigen around the operative excision area 7 days after the trabeculectomy surgeries. The results indicate that short-term use of some IOP-lowering drugs, such as latanoprost and unoprostone, may inhibit postoperative wound healing after glaucoma surgery.

Introduction

Glaucoma is one of the leading causes of permanent blindness. Trabeculectomy, a filtering surgery for glaucoma, is currently the most frequently performed surgical procedure in the treatment of this disease [1,2]. The surgery creates a block excision into the anterior chamber from under a partial-thickness scleral flap to form a filtering bleb. Aqueous humor flows through that passage from the anterior chamber to the subconjunctival space, to decrease the intraocular pressure (IOP). However, the prognosis and success rates for glaucoma surgery are largely dependent on wound healing and adhesion. Exaggerated wound healing, especially around the operative excision area, always results in the closure of the passage between the anterior chamber and the subconjunctival space therefore it leads to an obstruction of the outflow of aqueous humor and reduces the prognosis for surgical success [3].

The wound-healing process is a complex mechanism that involves proliferation at the wound site, cellular migration to the wound, and synthesis of new extracellular matrix components [4]. Studies have revealed that wound healing after glaucoma surgery consists mainly of fibroblast proliferation within the first 2 days and an increase in collagen components over time [5]. Some antineoplastic drugs have therefore been used to interfere with early postoperative fibroblast proliferation to prevent closure of the filtration fistula [6]. Many reports have, however, demonstrated that antineoplastic drugs are rather toxic to ocular tissues and cause many side effects, such as a high incidence of thin blebs, persistent hypotony, and choroidal detachment after trabeculectomy [7—9].

Studies have also shown that many IOP-lowering drugs have potent effects in inhibiting cellular proliferation in cultured Tenon's fibroblasts [10,11]. Moreover, some IOP-lowering drugs, such as latanoprost and unoprostone, reduce the release of extracellular matrix production by Tenon's capsule fibroblasts and modulate collagen turnover [12,13]. These effects may interfere with the early stage of wound healing after filtering surgery.

In the present study, we investigated the effects of several IOP-lowering drugs on the inhibition of wound healing, using cell culture and animal models. Initially, cultured Tenon's fibroblasts were used to screen the more inhibitive drugs in cellular proliferation, migration, and collagen synthesis. In the second step, the drugs that were selected from the cell culture were applied to the trabeculectomy wound area in rabbits. Finally, the PCNA numbers around the operative excision area after trabeculectomy surgery was immunohistochemically examined in order to select the best drug for wound healing inhibition.

Methods

Drugs preparation

Commercial IOP-lowering drugs including 0.5% levobunolol [Bunolgan, Allergan, 0.004% benzalkonium chloride (BAK)], 0.25% timolol (Timoptol, Merck Sharp & Dohme-Chibret, 0.0048% BAK), 0.03% bimatoprost (Lumigan, Allergan, 0.005% BAK), 0.004% travoprost (Travatan, Alcon, 0.015% BAK), 0.005% latanoprost (Xalatan, Pharmacia & Upjohn, 0.02% BAK), 0.12% isopropyl unoprostone (Rescula, Fujisawa, 0.015% BAK), 1% carteolol (Arteoptic, Otsuka, 0.005% BAK), 0.5% betaxolol (Betoptic, Alcon, 0.01% BAK), 1% brinzolamide (Azopt, Alcon, 0.01% BAK), 2% dorzolamide (Trusopt, Merck Sharp & Dohme-Chibret, 0.0075% BAK), 0.2% brimonidine (Alphagan, Allergan, 0.001% BAK), 1% pilocarpine (Spersacarpine, Dispersa, 0.01% BAK) and 0.1% dipivefrin (Propine, Allergan, 0.004% BAK) were available for this study. The BAK and diaminobenzidine were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals were obtained from Merck (Darmstadt, Germany).

Culture of rabbit Tenon's fibroblasts

Rabbit Tenon's capsule fibroblast tissue cultures were established according to the procedure described previously [11]. New Zealand albino rabbits were euthanized with ketamine (Parke-Davis, Ketalar, Pfizer). Dissection was performed under sterile conditions in a laminar-flow hood. The ocular Tenon's tissues were removed and minced into 1-mm cubes and plated in a culture flask. The culture medium, which contained RPMI-1640 (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO), 3.8mM L-glutamine (GIBCO) and 50 μg/mL gentamicin (GIBCO), was placed over the tissues. The tissues were cultured in a humidified 5% CO_2 incubator at 37°C. Fibroblasts usually appeared within 3—7 days. The cells used in this experiment were passage three to passage six.

[^{3}H]thymidine and [^{3}H]proline uptake

To estimate cellular proliferation and the rate of collagen formation, the uptake of [^{3}H]thymidine and [^{3}H]proline into cultured cells was measured following previously published procedures [14]. Cultured Tenon's fibroblasts were plated onto 12-well plates for at least 24 hours to allow for attachment. Cultured cells were incubated with serum-free RPMI-1640 medium containing various concentrations of drugs diluted from original commercial concentrations to 1/10, 1/100, and 1/1000 for 20 minutes at 37°C. Following

exposure to a particular drug, the cells were added to a fresh culture medium. Then, 1 μCi/mL of [^{3}H]thymidine (specific activity 15.0 Ci/mmol; New England Nuclear, Du Pont, Boston, MA, USA) or [^{3}H]proline (specific activity 112.0 Ci/mmol; New England Nuclear) was separately added into wells for 4 hours incubation, then uptake assays were stopped by rapid washing with ice-cold phosphate-buffered saline (PBS). The cells were lysed in 2% sodium dodecyl sulfate prior to quantification of radioactivity for [^{3}H]thymidine uptake. In the case of [^{3}H]proline uptake, cells were lysed in 2% sodium dodecyl sulfate and proteins were precipitated with 10% trichloroacetic acid. Precipitate was collected by filtration under vacuum onto glass fiber disks, which were washed twice with ice-cold 5% trichloroacetic acid and 100% ethanol, respectively. Radioactivity on the disks was measured by scintillation counting (Tri-Carb 2100TR Liquid Scintillation Counter, USA).

Cell migration

Cell migration was measured following previously published procedures [14]. Cells were seeded into the upper chambers of 12-well Transwell plates with 12-μm pores (Costar, Cambridge, MA, USA), allowing at least 24 hours for attachment. Cells in the upper chamber were incubated with serum-free RPMI-1640 medium containing various concentrations of drugs diluted from original commercial concentrations to 1/10, 1/100, and 1/1000 for 20 minutes at 37°C. The medium in the upper chamber was then removed and fresh serum-free medium was added to the upper and lower chambers. The assembled chambers were then incubated for 24 hours to allow the cells to migrate through the pores of the filter membrane. The filter and lower chamber were then fixed with formalin, stained with hematoxylin and air-dried. A microphotograph was taken at the focus level of the lower surface of the filter membrane and the lower chamber plate. Cell migration was measured by counting the number of spreading cells. Cells whose nuclei were present in the pores of the membrane were excluded.

Trabeculectomy in rabbits

The trabeculectomy experiment in rabbits was approved by the Institutional Animal Care and Use Committee of Kaohsiung Medical University. The trabeculectomies were performed by the same surgeon, according to the procedure described previously [15]. New Zealand albino rabbits, weighing 2.5—3.0 kg, were assigned to five groups: negative control (serum-free medium), 0.02% BAK diluted with serum-free medium, timolol, latanoprost, and unoprostone treatments. Each group consisted of 12 rabbits. All rabbits were anesthetized by intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine, and trabeculectomies were performed on their eyes (12 eyes of 12 rabbits). After creating a fornix-based conjunctival incision, a half-thickness scleral flap 3—4 mm wide was dissected into the clear cornea. Each group was administered a 25-μL solution, which was sterilized with a 0.2-μm filter in advance, administered under the scleral flap for 10 minutes. Then, a trapezoid block excision (about 1.5 × 2 mm) was created at the area to spur. Finally, the scleral flap and conjunctival wound were closed by suturing with 10-0 nylon. After the trabeculectomies, the respective solutions were administered topically, four times daily, on each surgical eye. Without any antibiotic or corticosteroid eye-drop application, the rabbits were euthanized, some on day 3 after the filtering surgery and some on day 7, with ketamine for histological and immunohistological examinations.

Histological preparation

The rabbits were histologically studied to examine the effects of various solutions on the inhibition of post-operative wound healing. At 3 and 7 days after surgery, six rabbits in each group were euthanized with ketamine. The eyeballs (6 eyes of 6 rabbits) were then nucleated and fixed in 3% cacodylate buffer overnight. The specimens were taken from the conjunctiva/Tenon's capsule incision containing the sclerostomy and suture areas for each eyeball. The segments were then dehydrated through a graded alcohol series and embedded in paraffin. Sections were vertically cut, with an operative excision area of 6 μm thickness, mounted on slides, and dried for histological staining. After removing the paraffin with CitriSolv solution and rehydration through a serial ethanol grade, one slice with an approximately maximal area in the operative excision area was selected for hematoxylin—eosin staining. Then, moving forward and backward, three more serial slices from each rabbit were selected for further immunohistochemical staining.

Immunohistochemical staining of proliferating cell nuclear antigen

Immunohistochemical staining was performed according to our previously published method [14]. Paraffin-embedded sections were de-paraffinized in CitriSolv solution and rehydrated through a graded alcohol series. Endogenous peroxidase activity was quenched by immersing the specimens in 2% H_2O_2 at room temperature for 5 minutes. The non-specific blots were blocked at room temperature for 10 minutes in PBS containing 5% normal goat serum. Sections were washed with PBS and rinsed with 0°C acetone for 20 seconds. Following a PBS washing, a biotinylated mouse antiproliferating cell nuclear antigen (PCNA) primary antibody (1:120; Zymed, San Francisco, CA, USA) was applied to the tissue in a humidified chamber at 37°C for 1 hour. After washing with PBS, tissues were further incubated with streptavidin—horseradish peroxidase (1:1000, Zymed) for 20 minutes at room temperature. The sections were then immersed in 0.2 mg/mL diaminobenzidine solution at room temperature for 5—10 minutes until a satisfactory color reaction was achieved. A qualitative analysis of the PCNA numbers around operative excision area was counted using Nikon light microscopy connected to a high-resolution television monitor.

Statistical analysis

Statistical analysis was performed for the cell culture and animal experiments by one-way analysis of variance,

followed with Dunnett's post-test, and are presented as mean ± standard deviation. In the cellular experiments, all data are presented as percentages of negative control, which was assumed as 100% response, and were collected from three experiments with triplicate determinations. The values from all the IOP-lowering drugs, diluted to 1/10, 1/100, and 1/1000 concentrations, were compared with BAK results at 0.002%, 0.0002%, and 0.00002%, respectively. In the animal study, the data were collected from six rabbits, with an average of six sections from each rabbit. The values were considered significant at $p < 0.05$.

Results

Effects of benzalkonium chloride

In the cellular study, only 0.002% BAK, which was regarded as the 1/10 group, caused a cytotoxic effect in [^{3}H]thymidine, proline uptake, and migration. BAK at 0.0002% and 0.00002% exhibited no significant toxic effects compared with the negative control value. In order to eliminate the potentially toxic effect of BAK in the drugs tested, the 1/10 diluted values of all the IOP-lowering drugs were compared with the result of 0.002% BAK, which was regarded as the 1/10 group. In the case of 1/100 and 1/1000, the diluted values of all drugs tested were compared with the 0.0002% and 0.00002% BAK values, respectively.

The effects of drugs on cellular [^{3}H]thymidine uptake

To estimate the effects of the IOP-lowering drugs, including betaxolol, timolol, bimatoprost, travoprost, latanoprost, unoprostone, carteolol, levobunolol, brinzolamide, dorzolamide, brimonidine, pilocarpine, dipivefrin, and BAK on cellular proliferation, [^{3}H]thymidine uptake was performed in the cultured cells. The effects of the IOP-lowering drugs on cellular proliferation are shown in Fig. 1. In all the drugs tested, the 1/10 diluted concentrations significantly inhibited [^{3}H]thymidine uptake compared with the negative controls, which were assumed to have 100% response. In the presence of 1/10 diluted concentrations of the various drugs, the cellular proliferation was reduced to 64% ± 3% by 0.002% BAK, 54% ± 2% by betaxolol, 51% ± 2% by timolol, 60% ± 2% by bimatoprost, 59% ± 3% by travoprost, 34% ± 1% by latanoprost, 11% ± 1% by unoprostone, 65% ± 2% by carteolol, 63% ± 2% by levobunolol, 61% ± 3% by brinzolamide, 59% ± 3% by dorzolamide, 65% ± 3% by brimonidine, 66% ± 2% by pilocarpine, and 59% ± 2% by dipivefrin, compared to the value of 15,308 ± 459 (DPM/mg total protein), which was assumed as a 100% response (Fig. 1A and B). Only betaxolol, timolol, latanoprost, and unoprostone at 1/10 diluted concentrations showed a significant decrease compared with the 0.002% BAK value, which was regarded as the 1/10 group.

Most of the IOP-lowering drugs at 1/100 and 1/1000 dilutions did not significantly decrease cellular [^{3}H]thymidine uptake, except for betaxolol, timolol, latanoprost, and unoprostone. The 1/100 diluted concentrations of betaxolol, timolol, latanoprost, and unoprostone inhibited cellular [^{3}H]thymidine to 85% ± 2%, 82% ± 3%, 65% ± 2%, and 34% ± 2%, respectively, compared with 0.0002% BAK, which had a value of 99% ± 2% (Fig. 1A). Moreover, only the 1/1000 diluted concentrations of latanoprost and unoprostone significantly inhibited cellular [^{3}H]thymidine to 81% ± 2% and 64% ± 2%, respectively, compared with 0.00002% BAK, which had a value of 100% ± 2% (Fig. 1A).

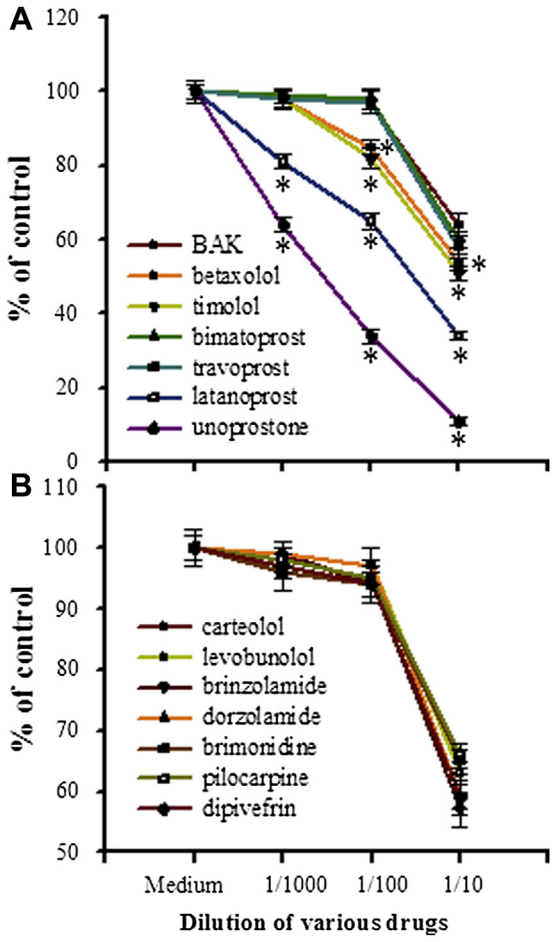

Figure 1. Dose-dependent curves of the IOP-lowering drugs and benzalkonium chloride (BAK) on cellular [^{3}H]thymidine uptakes in cultured Tenon's fibroblasts. Cells were exposed to various drugs, which were diluted to 1/10, 1/100, and 1/1000 from original concentrations. BAK was initially used with 0.02% as a maximal original concentration. The values from all the IOP-lowering drugs, diluted to 1/10, 1/100, and 1/1000 concentrations, were compared with BAK results at 0.002%, 0.0002%, and 0.00002%, respectively. The values were significantly different from corresponding BAK diluted group at $p < 0.05$.

The effects of drugs on cellular [^{3}H]proline uptake

To investigate collagen production, [^{3}H]proline uptake was performed in the cultured cells. The [^{3}H]proline uptake results were similar to the [^{3}H]thymidine uptake results. The 1/10 tested drugs significantly inhibited [^{3}H]proline uptake compared with the negative control, which was assumed as 100% response. The cellular [^{3}H]proline uptake for 0.002% BAK was reduced to 82% ± 3%, which was regarded as the 1/10

group (Fig. 2A). Of all the drugs tested, those diluted with 1/10 concentrations significantly inhibited [^{3}H]proline uptake compared with the negative control. At 1/10 diluted concentrations, only betaxolol, timolol, latanoprost, and unoprostone significantly decreased the 0.002% BAK value, which was regarded as the 1/10 group. In the presence of 1/10 diluted concentrations of the various drugs, cellular collagen production was reduced to 47% ± 2% by betaxolol, 32% ± 1% by timolol, 80% ± 2% by bimatoprost, 77% ± 3% by travoprost, 14% ± 1% by latanoprost, 9% ± 1% by unoprostone, 82% ± 3% by carteolol, 82% ± 3% by levobunolol, 77% ± 2% by brinzolamide, 79% ± 3% by dorzolamide, 78% ± 3% by brimonidine, 76% ± 3% by pilocarpine, and 80% ± 2% by dipivefrin, compared with the negative control value of 4607 ± 184 (DPM/mg total protein), which was assumed as 100% response (Fig. 2A and B). Most of the IOP-lowering drugs at 1/100 and 1/1000 dilutions did not decrease cellular [^{3}H]proline uptake, except for betaxolol, timolol, latanoprost, and unoprostone, compared with the respective BAK values. The 1/100 diluted concentrations of betaxolol, timolol, latanoprost and unoprostone significantly inhibited cellular [^{3}H]proline to 85% ± 3%, 80% ± 2%, 55% ± 2% ,and 39% ± 2%, respectively, compared with the 0.0002% BAK value of 100% ± 2% (Fig. 2A). Only 1/1000 diluted concentrations of latanoprost and unoprostone significantly inhibited cellular [^{3}H]thymidine to 78% ± 3% and 70% ± 2% compared with the 0.0002% BAK value of 100% ± 3% (Fig. 2A).

The effects of drugs on cellular migration

The dose-response curves of the drugs tested are shown in Fig. 3 for cellular migration. The results of cell migration were similar to [^{3}H]thymidine and [^{3}H]proline uptake, except for betaxolol. In the case of 0.002% BAK, which was regarded in the 1/10 group, cellular migration was

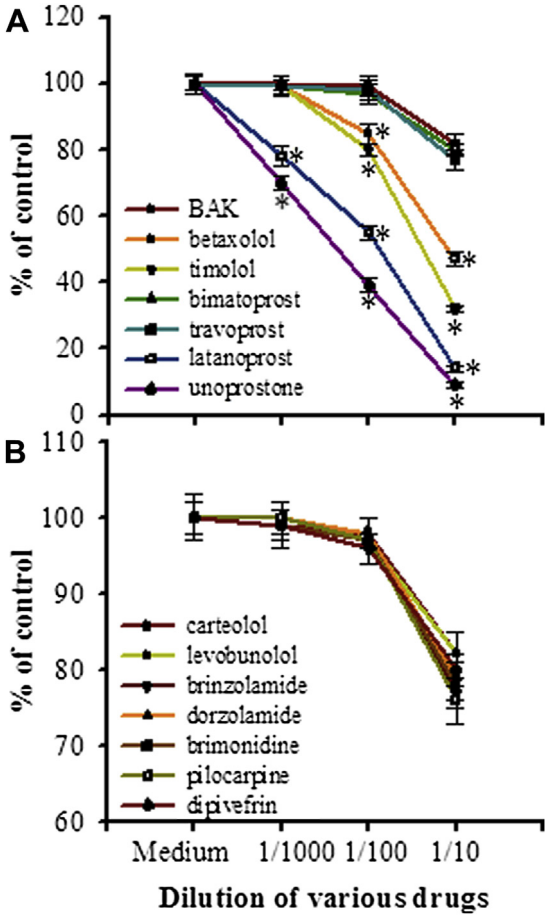

Figure 2. Dose-dependent curves of the IOP-lowering drugs and benzalkonium chloride (BAK) on cellular [^{3}H]proline uptakes on cultured Tenon's fibroblasts. Cells were exposed to various drugs, which were diluted to 1/10, 1/100, and 1/1000 from original concentrations. BAK was initially used with 0.02% as a maximal original concentration. The values from all the IOP-lowering drugs, diluted to 1/10, 1/100, and 1/1000 concentrations, were compared with BAK results at 0.002%, 0.0002%, and 0.00002%, respectively. The values were significantly different from corresponding BAK diluted group at $p < 0.05$.

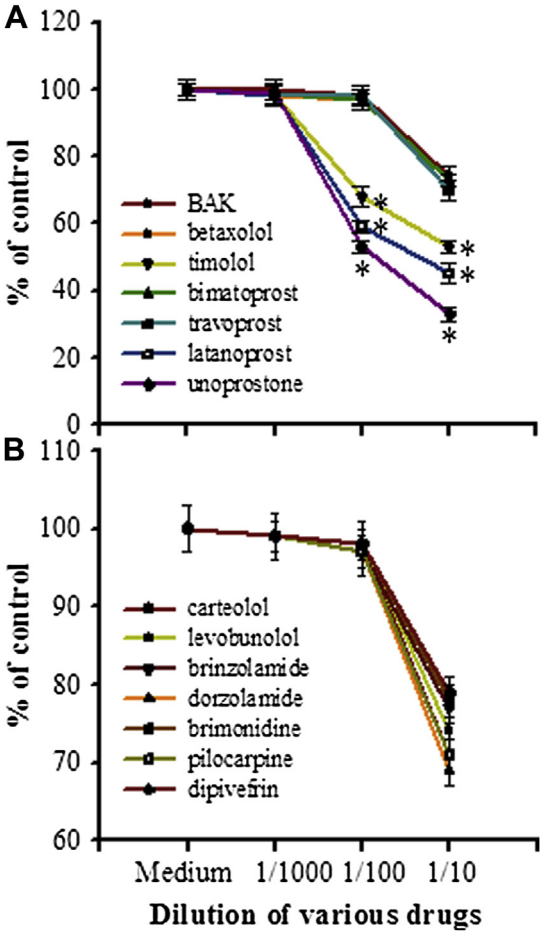

Figure 3. Dose-dependent curves of the IOP-lowering drugs and benzalkonium chloride (BAK) on cellular migration in cultured Tenon's fibroblasts. Cells were exposed to various drugs, which were diluted to 1/10, 1/100, and 1/1000 from original concentrations. BAK was used with 0.02% as a maximal original concentration. The values from all the IOP-lowering drugs, diluted to 1/10, 1/100, and 1/1000 concentrations, were compared with BAK results at 0.002%, 0.0002%, and 0.00002%, respectively. The values were significantly different from the corresponding BAK-diluted group at $p < 0.05$.

suppressed to 74% ± 3%, compared with the negative control value of 100% response. Most drugs exhibited no significant difference at 1/10 diluted concentrations compared with the 0.002% BAK value. The effect was inhibited to 73% ± 2% by betaxolol, 73% ± 2% by bimatoprost, 70% ± 3% by travoprost, 71% ± 2% by carteolol, 74% ± 3% by levobunolol, 77% ± 3% by brinzolamide, 69% ± 2% by dorzolamide, 78% ± 2% by brimonidine, 71% ± 1% by pilocarpine, and 79% ± 2% by dipivefrin (Fig. 3A and B).

Of all the drugs tested at 1/10 and 1/100 diluted concentrations, cellular migrations were only inhibited by timolol (to 53% ± 3% and 68 ± 2%), by latanoprost (to 45% ± 2% and 59% ± 3%), and by unoprostone (to 33% ± 2% and 53% ± 2%), respectively (Fig. 3A). Using the data from the cell cultures, we selected the three most effective drugs—timolol, latanoprost, and unoprostone—to be tested in the animal study.

The effects of drugs on proliferating cell nuclear antigen numbers after trabeculectomy in rabbits

The positive PCNA numbers were counted around the operative excision wound area in the five groups, which included negative control with serum-free medium, 0.02% BAK diluted with serum-free medium, timolol, latanoprost, and unoprostone groups 3 and 7 days after trabeculectomy in the rabbits. Three days after surgery, immunohistochemical staining of PCNA cells revealed that three drugs—timolol, latanoprost, and unoprostone—statistically decreased the numbers of PCNAs around the operative excision wound area compared with the control group (Fig. 4). The number PCNA cells with positive staining were 209 ± 31, 132 ± 17, 83 ± 8, and 34 ± 9 for 0.02% BAK, timolol, latanoprost, and unoprostone, respectively, compared with the negative control of 216 ± 26.

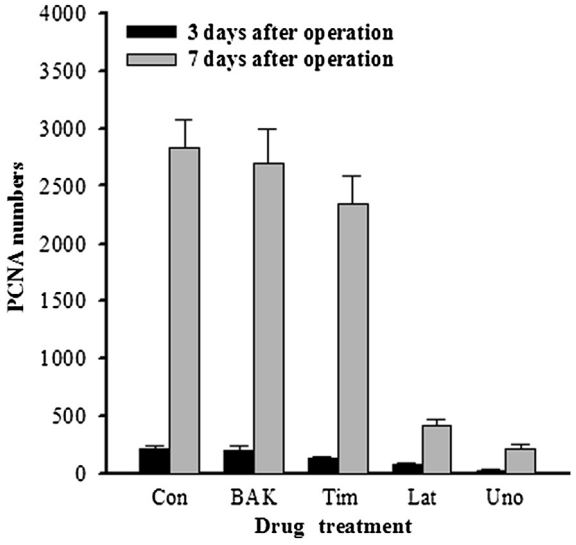

Figure 4. Numbers of proliferating cell nuclear antigen (PCNA) immunopositive cells around trapezoidal excision wound areas 3 and 7 days after trabeculectomy in rabbits. The cells were counted in the control (Con), 0.02% benzalkonium chloride (BAK), timolol (Tim), latanoprost (Lat) and unoprostone (Uno) groups. The data were collected from six rabbits, with an average of six sections from each rabbit. The values were significantly different from corresponding control group at $p < 0.05$.

Seven days after surgery, the 0.02% BAK and timolol groups exhibited no significant differences from the control group, with PCNA counts of 2698 ± 289 and 2348 ± 238, respectively. Only latanoprost and unoprostone statistically decreased the positive immunohistochemical PCNA numbers—427 ± 56 (latanoprost) and 216 ± 43 (unoprostone)—compared with the control number of 2827 ± 248. The typical morphological characteristics of hematoxylin and eosin staining (Fig. 5A, C and E) and immunopositive staining of PCNA cells (Fig. 5B, D and F) in the negative control and latanoprost- and unoprostone-treated sections 7 days after surgery are shown in Fig. 5. In the control eyes that underwent trabeculectomy, the number of immunoreactive PCNA cells increased after surgery. Seven days after surgery, in the control section, the hole created by the operative excision was almost obstructed and the PCNA cells had built up in the surrounding wound area (Fig. 5B). However, the numbers of immunoreactive cells in the latanoprost (Fig. 5D) and unoprostone-treated sections (Fig. 5F) were much lower than in the control section (Fig. 5B).

Discussion

In the present study, we determined whether the short-term application of some IOP-lowering drugs, such as latanoprost and unoprostone, could be utilized as wound-healing inhibitors to retard wound occlusion after glaucoma surgery. Until now, many commercial IOP-lowering drugs have been applied in the treatment of glaucoma. After glaucoma filtration surgery, IOP-lowering drugs are usually withdrawn, as the IOP has been decreased by the fistula that has been created. Even when IOP-lowering drugs are administered for a few days after surgery, they do not necessarily maintain bleb formation. Therefore, whether or not the IOP-lowering drug interferes the wound healing may be a matter of concern. In the present study, it was demonstrated that with short-term administration of latanoprost or unoprostone for a few days after filtering surgery, the wound area, especially the fistula entrance, may be maintained postoperatively for a longer period of time than without using an IOP-lowering drug.

When IOP-lowering drugs are introduced as a wound-healing inhibitor, the best time for the drug's application to inhibit extracellular matrix formation, cell migration, and fibroblast proliferation is during and after the operation. Wound healing in cellular proliferation after glaucoma filtering surgery happens as early as 24 hours post-operatively, and peak proliferation is during the first 5 days postoperatively [16]. Studies also have revealed that collagen is promptly enhanced during the first 7 days after filtering surgery [17]. Examination of PCNA, which is used as a marker of cell proliferation after filtration surgery, yields similar results in rabbit eyes [18]. Based on the data from our animal experiments, the number of immunoreactive

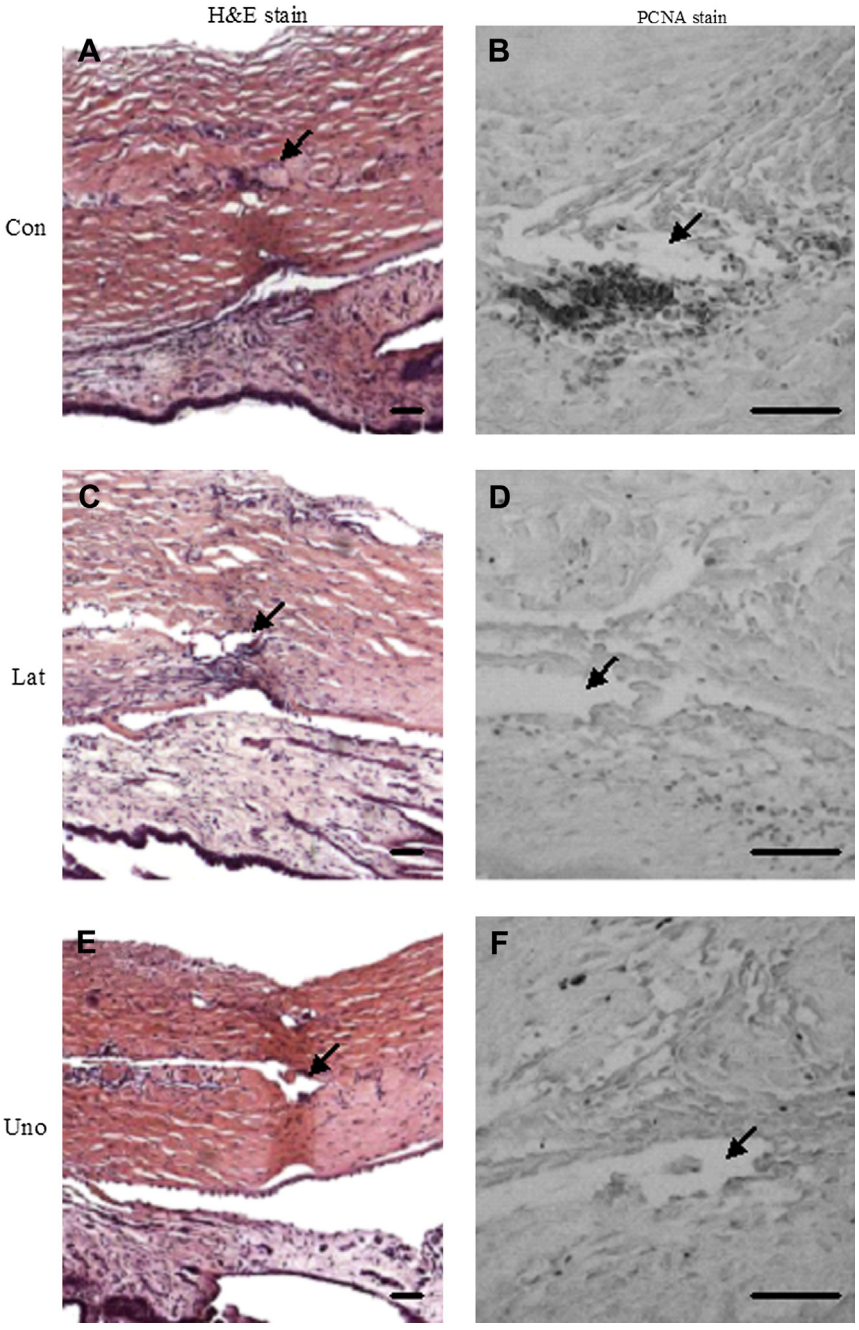

Figure 5. The typical morphological characteristics of hematoxylin and eosin staining and immunopositive staining of PCNA cells in the control and latanoprost- and unoprostone-treated sections 7 days after surgery in the rabbits. (A, C, E) Specimens were stained with hematoxylin—eosin (original magnification × 100) and (B, D, F) mouse anti-PCNA primary antibody (original magnification × 400) in the control (Con, A, B), latanoprost (Lat, C, D) and unoprostone (Uno, E, F) groups. Arrows indicate the trapezoidal excision wound areas.

PCNA cells in the control eyes promptly increased from three to seven after surgery, and the filtering site was almost completely obstructed by the 7th postoperative day. Thus, the critical time to inhibit wound healing is within the 1st week after filtering surgery.

The wound-healing process involves the production of extracellular matrix components such as collagen and cellular migration [4,19]. Based on the data from the cell culture, timolol, latanoprost, and unoprostone exhibited more inhibitory effects on collagen production and cellular migration than the other IOP-lowering drugs. Studies have revealed that timolol has an inhibitory effect on corneal epithelial migration [20]. However, timolol has less of an inhibitory effect than unoprostone on cell proliferation [21] and less than latanoprost on collagen degradation [13]. These effects may explain the results of the animal study, wherein timolol had less of an inhibitory effect than unoprostone or latanoprost.

Although many studies have suggested that therapy with topical IOP-lowering drugs may enhance the inflammatory cells and fibroblasts in the conjunctival tissues, these observations were acquired from long-term collections involving months and years of use [22,23]. Instead of long-term use, we utilized IOP-lowering drugs over the short term, during the critical period of wound healing, in order to prevent the formation of scarring after glaucoma surgery.

Recently, many prostanoids, such as bimatoprost, latanoprost, travoprost, and unoprostone have been used as IOP-lowering drugs in the treatment of glaucoma and ocular hypertension [24]. Based on the clinical data, the above four drugs exhibited potent IOP-lowering effects that were at least as effective as beta-blockers [25]. However, this study presents evidence for the divergence of the four prostanoids in wound-healing modulation. As of now, the exact mechanism of latanoprost and unoprostone in the inhibition of wound healing remains unclear. It is interesting to note that the effect of unoprostone is better than that of latanoprost, whether tested *in vitro* or *in vivo*. Similar studies have revealed that unoprostone has a more inhibitory effect on cellular proliferation than latanoprost in cultured conjunctival cells [26]. However, it may be explained that latanoprost displays a strong ability to decrease conjunctival collagen density [13], and it also causes the expression of transforming growth factor-β, which may stimulate cellular proliferation [27].

Commercial IOP-lowering drugs usually contain BAK as a preservative. Numerous studies have revealed that the BAK contained in IOP-lowering drugs, especially at high concentrations, has an inhibitory effect on the proliferation of Tenon's fibroblasts and may be responsible for ocular toxicities and inflammation with chronic use [10,28]. Despite this, our studies revealed that IOP-lowering drugs containing high concentrations of BAK were not greatly involved in the modulation of wound healing.

According to pharmacokinetic studies with local soaking of drugs under the sclera flap during trabeculectomy, the half-life of the drug is 18 minutes in the conjunctiva and 19.2 minutes in the sclera [29]. After topical administration of tritium-labeled latanoprost, about 7.7% of the applied dose is found in the cornea 15 minutes after drug administration [30]. Thus, we estimated the effects of various drugs in cell culture at 1/10, 1/00, and 1/000 diluted concentrations from an original dosage after 20 minutes' exposure.

In conclusion, the short-term applications of some commercial IOP-lowering drugs were introduced in the present study to suppress the wound-healing process after trabeculectomy. Although the exact mechanism by which latanoprost and unoprostone prevent wound healing after surgery is unknown, both drugs may potentially be used to substitute for some highly toxic, antineoplastic drugs that obstruct wound healing after glaucoma filtering surgery and, thus, increase the success rate.

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